In the specification:

Please replace the previously amended paragraph on page 8, lines 22-27, of the specification with the following currently amended paragraph.

> Fig. 1 shows the sequence alignment of previously known bacterial RNase P protein subunits using the ClustalW alignment program (Thompson et al., Nucleic Acids Research 22: 4673, 1994) and the alignment of the RNase P sequences of the present invention (SEQ ID NOs: 39-~~95~~ 91).

Please replace the paragraph on page 9, lines 1-23, of the specification with the following amended paragraph.

> Figs. 2A-2S shows the nucleic acid sequences (SEQ ID NOs 1-19) encoding the amino acid sequences (SEQ ID NOs 20-38) of the bacterial RNase P polypeptides of the invention. The nucleic acid and amino acid sequences were derived from the following pathogenic bacterial species: *Streptococcus mutans* (Fig. 2A; SEQ ID NOs: 1 and ~~18~~ 20, respectively); *Klebsiella pneumoniae* (Fig. 2B; SEQ ID NOs: 2 and ~~19~~ 21, respectively); *Salmonella paratyphi* A (Fig. 2C; SEQ ID NOs: 3 and ~~20~~ 22, respectively); *Pseudomonas aeruginosa* (Fig. 2D; SEQ ID NOs: 4 and ~~21~~ 23, respectively); *Corynebacterium diphtheriae* (Fig. 2E; SEQ ID NOs: 5 and ~~22~~ 24, respectively); *Chlamydia trachomatis* (Fig. 2F; SEQ ID NOs: 6 and ~~23~~ 25, respectively); *Vibrio cholerae* Serotype 01, Biotype El Tor, Strain N16961 (Fig. 2G; SEQ ID NOs: 7 and ~~24~~ 26, respectively); *Neisseria gonorrhoea* FA 1090 (Fig. 2H; SEQ ID NOs: 8 and ~~25~~ 27, respectively); *Neisseria meningitidis* Serogroup A, Strain Z2491 (Fig. 2I; SEQ ID NOs: 9 and ~~26~~ 28, respectively); *Streptococcus pyogenes* M1 (Fig. 2J; SEQ ID NOs: 10 and ~~27~~ 29, respectively); *Bordetella pertussis* Tohama I (Fig. 2K; SEQ ID NOs: 11 and ~~28~~ 30, respectively); *Porphyromonas gingivalis* W83 (Fig. 2L; SEQ ID NOs: 12 and ~~29~~ 31, respectively); *Streptococcus pneumoniae* Type 4 (Fig. 2M; SEQ ID NOs: 13 and ~~30~~ 32, respectively); *Clostridium difficile* 630 (Fig. 2N; SEQ ID NOs: 14 and ~~31~~ 33, respectively); *Camphylobacter jejuni* NCTC (Fig. 2O; SEQ ID NOs: 15 and ~~32~~ 34, respectively); *Bacillus anthracis* Ames (Fig. 2P; SEQ ID NOs: 16 and ~~33~~ 35, respectively); *Mycobacterium avium* 104 (Fig. 2Q; SEQ ID NOs: 17 and ~~34~~ 36, respectively); *Staphylococcus aureus* NCTC

8325 (Fig. 2R; SEQ ID NOs: 18 and ~~35~~ 37, respectively); and *Staplylococcus aureus* COL (Fig. 2S; SEQ ID NOs: 19 and ~~36~~ 38, respectively).

Please replace the paragraph on page 22, lines 10-21, of the specification with the following amended paragraph.

The preferred reaction buffer contains 50 mM Tris-HCl (pH 7.5), 100 mM ammonium chloride and 10 mM magnesium chloride. Concentrations of 10-100 mM, 25-500 mM and 1-100 mM of the above, respectively, can be substituted, as can other buffering agents such as MOPS or HEPES, or other monovalent cations, such as sodium or potassium. When the assay is run in either 96 ~~98~~ or 384-well polystyrene or polypropylene assay plates, there is a very significant decrease in the fluorescence intensity and polarization of the annealed substrate over time in the absence of enzyme. Various conditions have been tested to prevent the loss of signal with time. The preferred conditions include addition of 10-40 μg/ml carbonic anhydrase and 10-100 μg/ml polyC to the buffer. Other materials, such as, 0.5-5% glycerol, 10-100 μg/ml hen egg lysozyme, 10-50 μg/mL tRNA, 1-10 mM DTT, or 2-10 mM DTT can also be added to the buffer to prevent some loss of signal.

Kindly replace the Sequence Listing filed on January 10, 2003, with the amended Sequence Listing, filed with the Reply to Office Action on August 4, 2003.

In the claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) An isolated polypeptide comprising an RNase P consensus sequence wherein said polypeptide has RNase P protein activity, wherein said polypeptide is a bacterial polypeptide, and wherein said polypeptide is not a polypeptide from one of the following organisms: *Coxiella burnetii* (None Mile) U10529, *Rickettsia prowazekii* (Madrid E) AJ235272, *Neisseria meningitidis* (Z2491) AL162753, *Neisseria meningitidis* (MC58) AE002540, *Buchnera aphidocola* M80817, *Buchnera aphidocola* (SGS) AF008210, *Buchnera* sp. (APS) AP000398, *Haemophilus influenza* (RD KW20) U32848, *Escherichia coli* M11056, *Escherichia coli* (K-12) AE000394, *Proteus mirabilis* M58352, *Pseudomonas aeruginosa* (PAO1) AE004968, *Pseudomonas putida* P25752, *Salmonella typhi* (CT18), *Yersinia pestis* (Orientalis), *Xyelella fastidiosa* AE004083, *Campylobacter jejuni* (NCTC 11168) AL139076, *Helicobacter pylori* (26695) AE000645, *Helicobacter pylori* (J99) AE001557, *Micrococcus luteus* (S66) U64884, *Mycobacterium avium* (104) AF222789, *Mycobacterium bovis* (AF2122/97), *Mycobacterium leprae* (Lortist 6) L39923, *Mycobacterium tuberculosis* (H37Rv) AL021426 X92504, *Streptomyces bikiniensis* (Zorbonenis) M83112, *Streptomyces coelicolor* (A3(2)) M82836 AL049826 AF031590, *Bacillus halodurans* (C-125) AB013492, *Bacillus subtilis* (168) X62539 AL009126, *Mycoplasma capricolum* (mcs5) P14982, *Mycoplasma genitalium* (G-37) U39713, *Mycoplasma pneumoniae* (M-129) U00089, *Staphylococcus aureus* (ISP3) AF135268, *Ureaplasma urealyticum* (3/1) AE002158, *Pseudanabaena* sp. (PCC6903) AJ000513, *Synechocystis* sp. (PCC6803) X81989, *Borrelia burgdorferi* (212) Z12166,

Borrelia burgdorferi (B31) AE000783, *Treponema pallidum* (Nichols) P50069, *Chlamydia trachomatis* (serovar D) AE001351, *Chlamydia muridarum* (trachomatis MoPn) AE002160, *Chlamydophila pneumoniae* (CWL 029) AE001673, *Chlamydophila pneumoniae* (AR39) AE002251, *Deinococcus radiodurans* (R1) AE002049, *Thermotoga maritima* (MSB8) AAD36531, *B. burgdorferi*, *C. burnetii*, *C. pneumoniae*-2, *C. trachomatis*, *H. influenza*, *H. pylori*-48, *M. leprae*, *M. luteus*, *M. tuberculosis*-2, *M. bovis*, *Pseudanabaena*-6903, R. prowazeki, S. bikiniensis, *Synechocystis* 6803, *Staphylococcus aureus*, and *S. pneumoniae* ~~Coxiella burnetii (None Mile) U10529, Rickettsia prowazekii (Madrid E) AJ235272, Neisseria meningitidis (Z2491) AL162753, Neisseria meningitidis (MC58) AE002540, Buchnera aphidocola (unspecified) M80817, Buchnera aphidocola (SGS) AF008210, Buchnera sp. (APS) AP000398, Haemophilus influenza (RD KW20) U32848, Escherichia coli (unspecified) M11056, Escherichia coli (K-12) AE000394, Proteus mirabilis (unspecified) M58352, Pseudomonas aeruginosa (PAO1) AE004968, Pseudomonas putida (unspecified) P25752, Salmonella typhi (CT18) no accession number, Yersinia pestis (Orientalis) no accession number, Xyelella fastidiosa (unspecified) AE004083, Campylobacter jejuni (NCTC 11168) AL139076, Helicobacter pylori (26695) AE000645, Helicobacter pylori (J99) AE001557, Micrococcus luteus (S66) U64884, Mycobacterium avium (104) AF222789, Mycobacterium bovis (AF2122/97) no accession number, Mycobacterium leprae (Lortist 6) L39923, Mycobacterium tuberculosis (H37Rv) AL021426 X92504, Streptomyces bikiniensis (Zorbonenis) M83112, Streptomyces coelicolor (A3(2)) M82836 AL049826 AF031590, Bacillus halodurans (C-125) AB013492, Bacillus subtilis (168) X62539 AL009126, Mycoplasma capricolum (mcs5) P14982, Mycoplasma genitalium (G-37) U39713, Mycoplasma pneumoniae (M-129) U00089, Staphylococcus aureus (ISP3) AF135268, Ureaplasma urealyticum (3/1) AE002158, Pseudanabaena sp.~~

~~(PCC6903) AJ000513, Synechocystis sp. (PCC6803) X81989, Borellia burgdorferi (212) Z12166, Borellia burgdorferi (B31) AE000783, Treponema pallidum (Nichols) P50069, Chlamydia trachomatis (serovar D) AE001351, Chlamydia muridarum (trachomatis MoPn) AE002160, Chlamydophila pneumoniae (CWL 029) AE001673, Chlamydophila pneumoniae (AR39) AE002251, Deinococcus radiodurans (R1) AE002049, Thermotoga maritima (MSB8) AAD36531, B.burgdorferi, B.burgborferi partial, C.burnetii, C.pneumoniae-2, C.trachomatis, H.influenza, H.pylori-48, M.leprae, M.luteus, M.tuberculosis-2, M.bovis, Pseudanabaena-6903, R.prowazeki, S.bikiniensis, Synechocystis6803, Staphylococcus aureus, and S. pneumoniae~~].

2. (Original) The polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 20-38.

3-7. (Canceled)

8. (Original) A method of identifying an antibiotic agent, said method comprising:

i) obtaining an RNase P holoenzyme comprising the polypeptide of claim 1;

ii) contacting said holoenzyme with an RNase P substrate in the presence and in the absence of a compound; and

iii) measuring the enzymatic activity of said holoenzyme; wherein a compound is identified as an antibiotic agent if said compound produces a detectable decrease in said RNase P enzymatic activity as compared to activity in the absence of said compound.

9. (Previously presented) The method of claim 8, wherein said polypeptide has at least 95% identity to the corresponding twenty amino acids: R11, L12, F18, R46, G48, V51, K53, K54, A59, V60, R62, N63, K66, R67, R70, L80, D84, V86, L101, and L105 of *E. coli* RNase P.

10. (Original) The method of claim 8, wherein said activity is measured by fluorescence spectroscopy.

11. (Currently amended) The method of claim 8, wherein said RNase P substrate is fluorescently tagged $ptRNA^{Gln}$.

12. (Canceled)

13. (Previously presented) The method of claim 11, wherein said contacting is carried out in a buffer comprising 10-40 μg/ml carbonic anhydrase and 10-100 μg/ml polyC.

14. (Original) The method of claim 13, wherein said buffer further comprises at least one of the following:

0.5-5% glycerol;

10-100 μg/ml hen egg lysozyme;

10-50 μg/ml tRNA; or

1-10 mM DTT.

15. (Original) The polypeptide of claim 1, having 100% identity to the corresponding twenty amino acids: R11, L12, F18, R46, G48, V51, K53, K54, A59, V60, R62, N63, K66, R67, R70, L80, D84, V86, L101, and L105 of *E. coli* RNase P.

16. (Original) The polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence at least 95% identical to any one of SEQ ID NOS: 20-38.

17. (Currently amended) The polypeptide of claim 1, wherein said polypeptide, when combined with an RNA subunit to form an RNase P holoenzyme, has at least 20% of the enzymatic activity of an *E. coli* or *B. subtilis* RNase P holoenzyme, wherein said enzymatic activity is the hydrolysis of an RNase P substrate.

18. (Original) The method of claim 14, wherein said buffer comprises 2-10 mM DTT.

19. (Original) The method of claim 9, wherein said polypeptide has 100% identity to the corresponding twenty amino acids: R11, L12, F18, R46, G48, V51, K53, K54, A59, V60, R62, N63, K66, R67, R70, L80, D84, V86, L101, and L105 of *E. coli* RNase P.

20. (Original) The method of claim 8, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 20-38.

21. (Currently amended) The method of claim 8, wherein said polypeptide, when combined with an RNA subunit to form an RNase P holoenzyme, has at least 20% of the enzymatic activity of an *E. coli* or *B. subtilis* RNase P holoenzyme, wherein said enzymatic activity is the hydrolysis of an RNase P substrate.

22. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 20.

23. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 21.

24. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 22.

25. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 23.

26. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 24.

27. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 25.

28. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 26.

29. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 27.

30. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 28.

31. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 29.

32. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 30.

33. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 31.

34. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 32.

35. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 33.

36. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 34.

37. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 35.

38. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 36.

39. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 37.

40. (New) The polypeptide of claim 2, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 38.

In the drawings:

The attached sheet of drawings includes changes to Figure 1. This sheet replaces the original sheet of Figure 1.

REMARKS

The present invention provides RNase P polypeptides and methods for identifying antibiotics using these polypeptides.

Claims 1, 2, 8-11, and 13-21 were examined in this case. Claims 1, 2, 16, and 20 were rejected under 35 U.S.C. § 112, second paragraph, and claims 1, 2, 8-11, and 13-21 were rejected under 35 U.S.C. § 112, first paragraph. Claim 1 was rejected under 35 U.S.C. § 102, and claims 8, 10, 11, 13, and 14 were rejected under 35 U.S.C. § 103. Each of these rejections is addressed below in the order that it appears in the Office Action.

Support for the Amendments

Claim 1 has been amended to correct a typographical error in the accession number AJ000513 and to italicize the genus and species names. Support for this amendment is found in the Declaration of patent agent Dr. Vicki Healy, filed October 11, 2002, that lists the bacterial RNase P protein sequences that James Brown, the founder of the Ribonuclease P Database, indicated were available in his RNase P Database (RNase P) on March 1, 2000 and that are excluded from the polypeptides of the present invention (page 7, lines 17-22, of the specification). Claim 11 is amended to correct a typographical error. Amended claims 17 and 21 specify that the enzymatic activity of the *E. coli* or *B. subtilis* RNase P holoenzyme is the hydrolysis of an RNase P substrate (as disclosed, for example, on page 4, line 25 through page 5, line 8). Support for new claims 22-40 can be found in claim 2. The specification has been amended to correct typographical errors.

These amendments add no new matter.

Objection to the Drawings

The Examiner states that Figure 1 is objected to because the residues highlighted in black cannot be seen and the sequences are not labeled with their sequence identifiers (SEQ ID NOS: 39-91). The attached substitute drawings have been amended as requested by the Examiner and this objection can now be withdrawn.

Rejection of claims 1, 2, 16, and 20 under 35 U.S.C. § 112, second paragraph

Claim 1 was rejected under 35 U.S.C. § 112, second paragraph, for containing a typographical error in the accession number AJ000513 and for failure to italicize the genus and species names. Claim 1 has been amended as requested by the Examiner.

The Examiner further states that some of the bacterial RNase P polypeptides do not have accession numbers, despite the ability of bacteria to have more than one RNase P polypeptide. As stated in the Declaration of inventor Dr. Venkat Gopalan, filed with the Reply to Office Action on August 4, 2003, and attached hereto, there is only one RNase P polypeptide in each bacterial species. The different accession numbers for some RNase P polypeptide sequences is due to multiple deposits of the same sequence. In view of this amendment and clarifying remarks, this rejection should be withdrawn.

Claims 2, 16, and 20 were rejected under 35 U.S.C. § 112, second paragraph, for reciting non-elected embodiments of the invention. As indicated on page 2 of Office Action mailed August 29, 2001, the single species SEQ ID NO: 27 (*Neisseria gonorrhoeae*) is elected for prosecution on the merits. In the event that no generic claim is allowed, the claims shall be restricted to the elected species. Thus, applicants respectfully assert that claims 2, 16, and 20 need not be limited to

the elected species at this time. Such an amendment would potentially be appropriate when no generic claim is allowed. In the event that a generic claim is allowed, applicants assert that claims to the remaining species, which are written in dependent form, or which otherwise include all the limitations of the allowed generic claim should be considered as provided by 37 CFR § 1.141 and MPEP § 809.02(a). Applicants note that the Examiner has indicated that this rejection will be maintained until a generic claim is allowed or the claims are restricted to the elected species.

Rejection of claims 17 and 21 under 35 U.S.C. § 112, first paragraph

Claims 17 and 21 were rejected, under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner states that the specification "does not teach how much the activity is of the E. coli or B. subtilis Rnase P holoenzyme nor what the activity of the claimed polypeptides are." In view of the amendments, applicants respectfully disagree.

Applicants note that claims 17 and 21 have been amended to specify that the enzymatic activity of the *E. coli* or *B. subtilis* RNase P holoenzyme is the hydrolysis of an RNase P substrate. To this end, the specification teaches:

> [b]y "a polypeptide containing RNase P activity" is meant a polypeptide sequence that, when combined with an RNA subunit to form an RNase P holoenzyme, has 20%, 50%, 75%, or even 100% or more, of the enzymatic activity of an *E. coli* or *B. subtilis* RNase P holoenzyme. Preferably, the RNA subunit is from the same species when activity is tested. The enzymatic activity can be assessed, for example, by measuring *hydrolysis of an RNase P substrate*. Standard methods for conducting such hydrolysis assays are described herein and in the literature (see, e.g., Altman and Kirsebom, Ribonuclease P, *The RNA World*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999; Pascual and Vioque, Proc. Natl. Acad. Sci. 96: 6672, 1999; Geurrier-Takada et al., Cell 35: 849, 1983; Tallsjö and Kirsebom, Nucleic Acids

> Research 21: 51, 1993; Peck-Miller and Altman, J. Mol. Biol. 221: 1, 1991; Gopalan et al., J. Mol. Biol. 267: 818, 1997; and WO 99/11653).
>
> By "RNase P substrate" is meant a substrate in which hydrolysis by an RNase P holoenzyme requires the presence of the RNase P protein subunit. (page 4, line 25 through page 5, line 10, emphasis added)

Furthermore, the attached Gopalan Declaration states that a skilled artisan can easily measure the enzymatic activity of an RNase P holoezyme of interest by determining the rate of hydrolysis of an RNase P substrate using standard assays such as those disclosed on pages 19-23 of the specification or in any of the references provided in the passage cited above.

For example, the specification teaches the following exemplary assays and reaction buffers:

> [s]amples of the RNase P holoenzyme and the RNase P substrate are mixed, incubated, and measured for spectrophotometric polarization. When the substrate is cleaved by the RNase P holoenzyme, the 10-nucleotide 5'- leader sequence is released, which leads to a substantial change in the fluorescence polarization in the sample. (Campbell, I.D. & Dwed., R.A. pp. 91-125 The Benjamin/Cummings Publishing Company, Menlo Park, CA (1984); Lakowicz, J.R., Plenum Press, NY (1983)).
>
> The preferred reaction buffer contains 50 mM Tris-HCl (pH 7.5), 100 mM ammonium chloride and 10 mM magnesium chloride. Concentrations of 10-100 mM, 25-500 mM and 1-100 mM of the above, respectively, can be substituted, as can other buffering agents such as MOPS or HEPES, or other monovalent cations, such as sodium or potassium. When the assay is run in either 96 or 384-well polystyrene or polypropylene assay plates, there is a very significant decrease in the fluorescence intensity and polarization of the annealed substrate over time in the absence of enzyme. Various conditions have been tested to prevent the loss of signal with time. The preferred conditions include addition of 10-40 μg/ml carbonic anhydrase and 10-100 μg/ml polyC to the buffer. Other materials, such as, 0.5-5% glycerol, 10-100 μg/ml hen egg lysozyme, 10-50

μg/mL tRNA, 1-10 mM DTT, or 2-10 mM DTT can also be added to the buffer to prevent some loss of signal. (page 22, lines 3-21)

Such assays and preferred conditions can be used to readily determine if a candidate polypeptide has RNase P enzymatic activity.

Such standard assays can also be used to determine if a candidate RNase P polypeptide has at least 20% of the activity of *E. Coli* RNase P. Exhibit 1 of the Gopalan Declaration shows the results of an exemplary assay in which the activity of an RNase P polypeptide of interest (*N. gonorrhoeae*) was compared to that of *E. coli* RNase P to determine whether the activity of *N. gonorrhoeae* RNase P is at least 20% of the activity of *E. coli* RNase P. The reaction mixture included 1 nM RNase P RNA subunit, 1-5 nM RNase P protein subunit, 40 nM pre-tRNA substrate, 50 mM Tris-HCl, pH 7.5, 100 mM NH_4Cl, and 10 mM $MgCl_2$ at room temperature (22 °C). The reaction was allowed to proceed for 5-60 minutes.

The RNase P substrate was the 85-nucleotide pre-$tRNA^{Gln}$ from *Synechocystis*. The substrate was labeled with ^{32}P at one position at the 5'end. Therefore, only the full-length precursor of 85 nucleotides and the 5' processed leader sequence of 10 nucleotides are detectable on this autoradiogram. The 75-nucleotide mature product is not labeled, and therefore not detectable. Lanes labeled "E" contain samples from reactions incubated with the *E. coli* holoenzyme, and those labeled "N" contain samples from reactions incubated with the *N. gonorrhoeae* holoenzyme. Each pair of lanes represents a particular incubation time in minutes (denoted above the lanes).

It should be noted, however, that the assays provided require quantitation of the resulting signal or bands on the autoradiogram in order to determine and compare the values for *E. coli* RNase P activity and the candidate RNAse P polypeptide activity. Quantitation of autoradiogram bands is a standard art-known

procedure which can be accomplished either by scanning and determining the density values for each of the bands on an autoradiogram or by direct exposure of the membrane to a phosphorous screen in a phosphoimager that allows for quantitation of the radioactive signal itself. These methods provide *relative* and not *absolute values* depending on several variables including the exposure time of the gel to the autoradiogram or of the membrane to the phosphoimager. These methods allow for a direct comparison of the *relative activity* of one or more RNase P polypeptides of interest to that of *E. coli* or *B. subtilis* RNase P in order to determine if an RNase P polypeptide of interest has at least 20% of the enzymatic activity of the *E. coli* or *B. subtilis* RNase P. These assays do not provide *an absolute value* for the enzymatic activity of *E. coli* or *B. subtilis* RNase P. Therefore the Examiner's request that applicants provide *a specific value* for the activity of the *E. coli* or *B. subtilis* RNase P holoenzyme is inappropriate and should be withdrawn.

As one skilled in the art can readily compare the activity of an RNase P polypeptide of interest to that of *E. coli* or *B. subtilis* RNase P, this rejection can now be withdrawn.

Rejection of claims 1, 2, 8-11, and 13-21 under 35 U.S.C. § 112, first paragraph

Claim 1, 2, 8-11, and 13-21 were rejected, under 35 U.S.C. § 112, first paragraph, for lack of enablement. In particular, the Examiner states that while the specification is enabling for the 19 different RNase P polypeptides described in SEQ ID NOs: 20-38, it does not teach "any and all polypeptide sequences" that are not listed in the internet RNase P database (as of March 3, 2000) and therefore lacks enablement. The Examiner also states that applicants have not enabled the claims drawn to polypeptides having 95% or 100% identity with 20 amino acids of *E. coli* RNase P because applicants have not demonstrated that these polypeptides

have RNaseP activity nor have they taught how to make these polypeptides. Applicants respectfully disagree.

The standard for enablement in the biotechnology arts has been set forth in *In re Wands* (858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988)). *Wands* holds that an invention is enabled so long as the teaching of the specification provides the invention without undue experimentation. *Wands* states that:

> the test [for determining whether experimentation is undue] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed (emphasis added).

Furthermore, the Federal Circuit has long held that it is not necessary for all possible embodiments of a claim to be operative in order for that claim to be enabled. *See Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 224 U.S.P.Q. (Fed. Cir. 1984). The proper test of enablement is "whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with the information known in the art without undue experimentation." *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d. 1318 (Fed. Cir. 1985).

Applying these standards to the present case, it is clear that the present specification satisfies the proper test of enablement as outlined above. As the Examiner acknowledges, the specification is enabling for the 19 different RNase P proteins identified in SEQ ID NOs: 20-38[1]. The specification provides a clear description of the steps used to identify these 19 polypeptide sequences (pages 10-17). Each of these polypeptides was identified through a BLAST search of

1 Applicants again note that the election of the single species (SEQ ID NO: 27) is for prosecution on the merits in the event that no generic claim is allowed.

bacterial genomes using query sequences from known bacterial RNase P polypeptides. The specific parameters for the search and the RNase P consensus sequence used to further refine the search are provided in the specification (see, for example, page 10, line 4 to page 11, line 25). One of ordinary skill in the art can readily use these same methods to identify any and all RNase P polypeptides of the invention.

The application discloses 19 separate RNase P polypeptides sequences and at least one previously described RNase P sequence (*Staphylococcus aureus*, page 12, lines 25 to 28) that were identified using these methods. Given that these methods were used to identify a large number of exemplary RNase P polypeptides, it would not be considered undue experimentation to use the described methods to identify additional RNase P polypeptides. As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. § 112 is satisfied. *In re Fisher* 427 F.2d 833, 839 166 U.S.P.Q. 18, 24 (CCPA 1970).

> It is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art. However, there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the invention as broadly as it is claimed. This means that the disclosure must adequately guide the art worker to determine, without undue experimentation, which species among all those encompassed by the claimed genus possess the disclosed utility. *In re Vaeck*, 947 F.2d 488, 496, 20 U.S.P.Q.2d 1438, 1445 (Fed. Cir. 1991).

Applicants assert that both illustrative examples and terminology, as described above, are provided in the specification. Furthermore, clear guidance is

also provided to allow the art worker to determine which species possesses the disclosed utility, namely RNase P activity.

At the time of filing, a skilled artisan, using no more than routine experimentation and the teachings of the present specification, could easily identify other polypeptides having an RNase P consensus sequence and assay them for RNase P activity using standard techniques. For example, the specification teaches;

> [b]y "an RNase P consensus sequence" is meant a sequence which, when aligned to the *E. coli* RNase P sequence using the ClustalW program and performing a comparison of the specified amino acid sequences, shows conservation of at least nine of the following specified 20 amino acid residues in the *E. coli* RNase P protein subunit: R11, L12, F18, R46, G48, V51, K53, K54, A59, V60, R62, N63, K66, R67, R70, L80, D84, V86, L101, and L105. Preferably, the consensus sequence conserves at least 13 of the 20 residues. It is also preferred that the aligned consensus sequence contain at least seven of the following subset of nine amino acid residues in the *E. coli* RNase P protein: F18, R46, K53, A59, R62, N63, K66, R67, R70, more preferably, at least eight of the amino acids, and, most preferably, all nine amino acids of the above subset. For the purpose of determining identity in the present invention, identity of amino acids or other than those for which the amino acid is specified in the consensus sequence are ignored in the comparison when calculating identity of nucleic acids encoding an RNase P consensus sequence degenerate codons encoding the designated amino acid are treated as identical (page 7, lines 1-16).

Based on these teachings, one skilled in the art can readily determine whether a polypeptide has an RNase P consensus sequence and if the polypeptide has RNase P activity using any of the assays taught in the specification (see, for example, pages 5 and pages 19-23).

As an example, Exhibit 2 of the attached Declaration of Dr. Gopalan illustrates the ability of recombinant *N. gonorrhoeae* RNase P to cleave an 85-nucleotide pre-$tRNA^{Gln}$ substrate using the methods described in the specification.

In another example, Exhibit 3 illustrates the ability of recombinant *Porphyromonas gingivalis* RNase P to cleave pre-tRNA^{Gln} using similar reaction conditions. These results confirm that polypeptides with the RNase P consensus sequence have RNase P activity.

Using the methods described in the specification, a skilled artisan can readily test polypeptides having 95% or 100% identity with the 20 specified amino acids of *E. coli* RNase P for RNase P activity. The present situation is, in all important aspects, indistinguishable from the facts in *Wands* in which the Federal Circuit held that the applicant's claim was enabled, despite the necessity for screening, because the process of screening was straightforward.

In conclusion, the facts in the present case clearly satisfy the proper test for enablement set forth by the courts and compel withdrawal of the 35 U.S.C. § 112, rejection.

Applicants also point out that, to sustain an enablement rejection, the Office has the initial burden to establish a reasonable basis to question the enabling nature of an applicant's specification. Thus, in a case in which the PTO questions the enablement of a claim, the CCPA, in *In re Marzocchi*, 439 F.2d 220, 169 U.S.P.Q. 367, 369 (CCPA 1971) has stated that:

> a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support (emphasis added).

Applicants note that for all of the aforementioned reasons no scientific evidence currently made of record in this case establishes a basis for doubting the objective truth of the statements found in applicants' specification regarding

enablement with respect to isolating RNase P polypeptides falling within applicants' claims and determining whether such polypeptides possess RNase P activity.

Rejection of claim 1 under 35 U.S.C. § 102

Claim 1 was rejected, under 35 U.S.C. § 102(b), as being anticipated by Gress (WO 99/11653), Guth (EP 0 811 688), Altman (The RNA World, 2:1155-1184, 1999, and FASEB Journal, 7:7-14, 1993), Frank (Annu. Rev. Biochem., 67:153-180, 1998), Gopalan (J. Mol. Biol., 267:818-829, 1997), Pace (J. Bacteriol., 177:1919-1928, 1995), Pascual (Proc. Natl. Acad. Sci. USA, 96:6672-6677, 1999), or Peck-Miller (J. Mol. Biol., 221:1-5, 1991). The Examiner maintains this rejection because applicants have not shown that the RNase P in the database is that excluded by the instant claim.

These above-cited references focus on the following bacterial RNase P subunits or complexes from the following bacteria: *S. pneumoniae* (Gress), *Staphylococcus aureus* (Guth), *E. coli* (Altman, 1999), *E. coli*, *Bacillus subtilis*, *Proteus mirabilis*, *Streptomyces bikiniensis*, and *Micrococcus luteus* (Altman, 1993), *E. coli*, *Bacillus subtilis* (Frank), *E. coli, Buchnera aphidocola, Coxiella burnetii, Haemophilus influenzae, Proteus mirabilis, Pseudomonas putida, Mycoplasma capricolum, Mycobacterium leprae, Micrococcus luteus, Streptomyces coelicolor*, and *Bacillus subtilis* (Gopalan), *E. coli* and *Bacillus subtilis* (Pace), *E. coli* and *Synechocystis* (Pascual), and *E. coli* (Peck-Miller). RNase P polypeptides from these bacteria have been excluded from amended claim 1. As described above and in the attached Declaration of inventor Dr. Venkat Gopalan, there is only one RNase P polypeptide in each bacterial species. The different accession numbers for some RNase P polypeptide sequences is due to multiple deposits of the same sequence. The amended claim 1 specifically excludes the only RNase P

polypeptide known for each of the above-mentioned bacterial species. In view of this amendment and these clarifying remarks, it should now be clear that the RNase P polypeptides disclosed in the above-mentioned references do not fall within the scope of amended claim 1. This rejection may now be withdrawn.

Rejection of claims 8, 10, 11, 13, and 14 under 35 U.S.C. § 103(a)

Claims 8, 10, 11, 13, and 14 were rejected, under 35 U.S.C. § 103(a), as being unpatentable over Potuschak (Nucl. Acids Res. 21:3229-3243, 1993), Mikkelsen (Proc. Natl. Acad. Sci., USA, 96:6155-6160, 1999), or Schroeder (EMBO J., 19(1):1-9, 2000). The Examiner states that it would have been obvious to identify an antibiotic agent by seeing if it decreased the activity of RNase P on a RNase P substrate in view of the references above.

For the reasons provided below, applicants contend it would not have been prima facie obvious to use a bacterial RNase P holoenzyme to identify antibiotic agents.

To establish a *prima facie* case for obviousness, the Office must establish that one skilled in the art would have been motivated to combine the prior art references and that there would have been a reasonable expectation of success. "Both the suggestion and the expectation of success must be founded in the prior art, not in the applicant's disclosure." *In re Dow Chem. Co.*, 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). As the Federal Circuit recently observed:

> A critical step in analyzing the patentability of claims pursuant to section 103(a) is casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field . . . . Most if not all inventions arise from a combination of old elements . . . . Thus, every element of a claimed invention may often be found in the prior art . . . . However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the

> whole claimed invention . . . . Rather, to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant.

In re Kotzab, 217 F.3d 1365, 1369-70, 55 U.S.P.Q.2d 1313, 1316 (Fed. Cir. 2000) (citations omitted). The evidence of a suggestion, teaching or motivation to combine "must be clear and particular." *In re Dembiczak*, 175 F.3d 994, 999, 50 U.S.P.Q.2d 1614, 1616 (Fed. Cir. 1999). In the present case, the Examiner has not met the standard outlined above.

Potuschak explores the similarities between two mouse ribonucleoproteins, RNase MRP and RNase P using two different antibiotics, puromycin and cycloheximide. Potuschak demonstrates that puromycin can inhibit the activity of both mouse enzymes while cycloheximide has no effect. Potuschak also uses *E. coli* RNase P holoenzyme purified from a crude bacterial extract to demonstrate that *E. coli* RNase P can cleave the substrate for RNase MRP and again demonstrates that the two enzymes are similar in their ability to cleave the same substrate.

The Potuschak reference does not teach the use of a bacterial RNase P holoenzyme to identify antibiotic agents, nor does it render such a method obvious.

The Schroeder reference is a review that covers the modulation of RNA function by antibiotics that bind RNA such as the aminoglycoside family of antibiotics. In this review the authors refer to the Mikkelsen reference also cited by the Examiner for this obviousness rejection. The Mikkelsen reference demonstrates the ability of aminoglycosides to interact with and inhibit the enzymatic activity of the RNA subunit of the *E.Coli* RNase P holoenzyme. The Mikkelsen reference is directed to understanding how a particular family of antibiotics functions in relation to various RNA molecules. The Mikkelsen reference does not suggest using an

RNase P holoenzyme to identify antibiotic agents, nor does it render such a method obvious.

In addition, claim 8 of the present application requires as a first step obtaining an RNase P holoenzyme comprising a bacterial polypeptide subunit described in claim 1. Claim 1 is directed to novel bacterial polypeptides having RNase P protein activity and specifically excludes *E. coli* RNase P. The Schroeder and the Mikkelsen references only describe *E. coli* RNase P and not the RNase P polypeptides of the invention, therefore these references, which do not describe the polypeptides of the invention can not render the invention obvious.

Furthermore, the claimed method is directed to a method for using the polypeptides of the invention for identifying an antibiotic agent. The cited references not only fail to describe the novel polypeptides of the invention but also fail to describe a method using such polypeptides to identify an antibiotic agent. One of the criteria required to establish a *prima facie* case of obviousness is that the prior art reference must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck, supra*. The Schroeder and the Mikkelsen references do not describe the polypeptides of the invention nor do they describe the use of such polypeptides in a screen for antibiotic agents. These references do not teach or suggest all the claim limitations and therefore cannot render the claimed invention obvious.

In conclusion, there is no motivation to combine the teachings of Potuschak, which demonstrates the function of two structurally similar mouse enzymes, with Mikkelsen, which demonstrates the function of a particular family of antibiotics on RNA, to render obvious the use of bacterial RNase P holoenzymes to identify antibiotic agents as claimed in the present invention. This rejection should be withdrawn.

CONCLUSION

In summary, applicants submit that the claims are now in condition for allowance, and such action is respectfully requested.

Enclosed is a Petition to extend the period for replying to the final Office action for three months, to and including October 4, 2003, and a check in payment of the required extension fee. Also enclosed is a check for $117.00 for the excess claims.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: October 3, 2003

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